PROTECTIVE EFFECTS OF SELENIUM, CALCIUM, AND MAGNESIUM AGAINST ARSENIC-INDUCED OXIDATIVE STRESS IN MALE RATS

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Inorganic arsenic is a potent carcinogen and environmental pollutant. More than one hundred million people are reported to be exposed to elevated concentrations of arsenic mainly via drinking water. Essential trace elements can affect toxicity of metals by interacting with metals at the primary site of action and can also modify the body's response to toxic metals by altering their metabolism and transport. This study investigates the effects of concomitant administration of selenium, magnesium, and calcium with arsenic on blood biochemistry and oxidative stress. Selenium was the most effective in reducing arsenic-induced inhibition of blood δ-aminolevulinic acid dehydratase (ALAD) activity and liver oxidative stress. Calcium and magnesium also showed favourable effects on haematological and other biochemical parameters. Because selenium was the most effective, it should be added to chelation therapy to achieve the best protective effects against arsenic poisoning in humans.

KEY WORDS: *aminolevulinic acid dehydratase, glutathione, reactive oxygen species, thiobarbituric acid reactive substances, trace elements*

Arsenic (As) is a widespread environmental toxicant that may cause neuropathy, skin lesions, vascular lesions, and cancer in chronic exposure (1, 2). It exists in the inorganic and organic form and in different oxidation states (-3, 0, +3, +5). Exposure to arsenic is usually through drinking water containing naturally high amounts of inorganic arsenic. Chronic arsenic poisoning has been widely reported in the general population worldwide. Recent studies have suggested that its toxicity is owed to the production of reactive oxygen species (ROS) (3, 4), which include hydrogen peroxide and free radicals such as superoxide anions. Sodium arsenite has been shown to enhance haeme oxygenase production; an indicator of oxidative stress. Sodium arsenate has the ability to substitute phosphate in enzyme-catalysed reactions,

where it can disrupt normal function of the enzymes (5).

Selenium is an essential component of glutathione peroxidase, an antioxidant enzyme, which plays a critical role in the body's antioxidant defence against oxidative damage by preventing the production of ROS (6). Arsenic and selenium are metalloids with similar chemical properties, but with markedly different biological effects. Selenium is known to protect against arsenic toxicity (7, 8). Glutathione (GSH) acts as a nucleophilic scavenger of numerous toxic compounds and their metabolites via enzymatic and chemical mechanisms and plays an important role in protecting against oxidative damage caused by ROS (9, 10). GSH can react non-enzymatically with ROS or can act as a substrate in GSH peroxidase-mediated

destruction of hydroperoxides. GSH depletion can impair a cell's defence against the toxic action of many compounds and may lead to cell injury and death (10).

The aim of this study was to see the effects of selenium, magnesium, or calcium co-administered with arsenic on biochemical indicators such as haem synthesis, blood, liver, and kidney oxidative stress, liver injury, and blood and tissue arsenic concentration in male rats.

MATERIALS AND METHODS

All experiments were performed on 30 male Wistar rats, each weighing 50 g to 70 g. The animals were obtained from Defense Research and Development Establishment (DRDE), Gwalior, India. All experiment protocols were approved by DRDE's ethics committee. Before the experiment, the animals were housed in stainless steel cages (six per cage) in an air-conditioned room with temperature maintained at (25±2) °C and accustomed to 12-hour light and dark cycle (light from 6:00 h to 18:00 h) for seven days. The rats received standard rat chow diet (Amrut Feeds, Pranav Agro, New Delhi, India) and water *ad libitum* throughout the experiment.

The rats were divided into five groups of six animals each and were receiving the following treatment for 3 weeks:

Group 1: drinking water

Group 2: 50 mg L^{-1} of sodium arsenite dissolved in drinking water

Group 3: 50 mg L^{-1} of sodium arsenite + 6.3 µmol L^{-1} of sodium selenite by gavage (2 mL kg^{-1}) once a day.

Group 4: 50 mg L^{-1} of sodium arsenite + 20 mmol L^{-1} of magnesium sulphate by gavage (2 mL kg^{-1}) once a day.

Group 5: 50 mg L^{-1} of sodium arsenite + 20 mmol L^{-1} of calcium sulphate by gavage (2 mL kg^{-1}) once a day.

The doses for arsenic and trace elements were based on previous studies (3, 11). All animals were anaesthetised with light ether before sacrificing. Whole blood was collected by cardiac puncture and placed in heparinized vials. The liver and the kidneys were removed, rinsed in cold saline, blotted, and weighed before biochemical tests.

The activity of blood δ-aminolevulinic acid dehydratase (ALAD) was assayed according to Berlin and Schaller (12), while white blood cells (WBC), red blood cells (RBC), haematocrit (Hct), haemoglobin (Hb), mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) were measured using a haematology analyser (Sysmex K4500, USA). Blood GSH concentration was determined using a modified method by Jollow et al. (13). Liver and kidney GSH and GSSG (oxidised GSH) levels were determined with a spectrofluorometer (Shimadzu RF 5000, Japan) using the method of Hissin and Hilf (14). Tissue lipid peroxidation was measured using the method of Ohkawa et al. (15). ROS was determined using the method of Socci et al. (16). The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were determined using the method described by Reitman and Frankel (17).

Blood, liver, kidney, and brain arsenic were measured after wet acid digestion using a Microwave Digestion System (CEM, USA, model MDS-2100). Arsenic was estimated using a Hydride Vapor Generation System (Perkin Elmer model MHS-10) fitted with an atomic absorption spectrophotometer (AAS, Perkin Elmer model Analyst 100).

Data are expressed as mean ± SE (standard error). Mean values were compared between the group using one-way ANOVA and Tukey's test. Statistical significance for differences between the unexposed and arsenic-exposed groups (with or without micronutrient) was set at $P<0.05$.

RESULTS

Exposure to arsenic alone led to a significant decrease in blood ALAD activity and in GSH levels, while ROS showed a significant increase. Co-administration with Se (6.3 µmol L^{-1}) showed a significant improvement in ALAD, ROS, and GSH levels, while co-administration with calcium or magnesium had only a marginal effect on these parameters (Table 1).

In the kidneys, exposure to arsenic alone led to a significant increase in ROS, but GSH, GSSG, and thiobarbituric acid reactive substances (TBARS) levels did not change significantly. Co-administration with selenium, calcium, or magnesium showed no significant effects on any of these parameters (Table 2).

Table 3 shows biochemical changes in the liver which indicate oxidative stress. ROS, TBARS and

GSH levels significantly increased in arsenic-exposed animals, while liver GSSG increased marginally. Co-administration with selenium significantly counteracted arsenic effects, but magnesium and calcium had no effect on any of these parameters.

Exposure to arsenic alone significantly increased serum AST and ALT, while co-administration with selenium, calcium, or magnesium prevented this increase.

Tables 1-3 show arsenic concentrations in the blood, liver, and kidneys of animals exposed to arsenic alone or in combination with selenium, magnesium, or calcium. Co-administration with magnesium and selenium had a marginal beneficial effect on blood and liver arsenic concentrations, while calcium had no effect at all.

DISCUSSION

Arsenic in water is a result of natural and human activities. It is believed that nearly one hundred million people are at risk of arsenic exposure via drinking water. The arsenic issue is perhaps the most distinct in Bangladesh and West Bengal, as millions of hand-pumped tube wells yield drinking water with arsenic concentrations above 10 μg L^{-1}, which is the World Health Organization drinking water guideline (18-20). In West Bengal, India, arsenic concentrations in some tube wells go as high as 3400 μg L^{-1} (21).

This study has investigated the effects of arsenic on biochemical parameters indicative of changes in haeme biosynthesis, liver and kidney oxidative stress, and liver damage, and has investigated the ability of

Table 1 *Haematological effects of selenium, magnesium, and calcium in arsenic-exposed male rats*

Animal group	ROS / nmol mL^{-1}	GSH / mg mL^{-1}	ALAD / nmol mL^{-1}	Blood As / ng dL^{-1}
Negative control	0.011±0.004 (0.009 to 0.013)	6.57±0.11 (6.31 to 6.71)	29.94±1.2 (25.72 to 36.12)	0.16±0.02 (0.13 to 0.19)
As	0.028±0.003† (0.026 to 0.033)	4.88±0.11† (4.59 to 5.14)	19.52±1.4† (17.03 to 22.14)	8.18±0.12† (7.65 to 8.56)
As+Se	0.018±0.001‡ (0.017 to 0.019)	5.89±0.31‡ (4.90 to 6.18)	26.69±1.6 (22.97 to 30.23)	5.87±0.23‡ (5.34 to 6.35)
As+Mg	0.014±0.002 (0.012 to 0.16)	5.18±0.11† (4.91 to 5.47)	21.05±0.46† (20.19 to 22.14)	8.37±0.38† (8.01 to 8.90)
As+Ca	0.014±0.001 (0.013 to 0.015)	4.67±0.22† (4.54 to 4.77)	23.79±0.42† (23.08 to 25.02)	7.27±0.44† (6.45 to 7.94)

ROS - reactive oxygen species measured in RBC in the first minute; GSH - glutathione; ALAD - δ-aminolevulinic acid dehydratase measured in RBC in the first minute; values are expressed as mean ± SE (N=6); range values are in parenthesis
† - significantly different from all other values; ‡ - significantly different from unmarked values.

Table 2 *Effects of selenium, magnesium, and calcium on arsenic-induced oxidative stress parameters in rat kidney*

Experimental group	TBARS / μg g^{-1}	ROS / nmol mL^{-1}	GSH / μmol g^{-1}	GSSG / μmol g^{-1}	Kidney As / ng g^{-1}
Negative control	4.8±0.96 (3.4 to 7.5)	1.5±0.43 (0.76 to 2.3)	3.3±0.47 (2.5 to 3.6)	2.7±0.14 (2.3 to 3.0)	0.05±0.01 (0.03 to 0.06)
As	5.0±0.23 (4.3 to 5.2)	2.9±0.22† (2.4 to 3.48)	3.0±0.10 (2.7 to 3.2)	2.9±0.11 (2.4 to 3.5)	1.19±0.34† (0.64 to 1.65)
As+Se	4.2±0.68 (2.6 to 5.4)	2.9±0.63† (1.5 to 4.1)	3.4±0.47 (3.0 to 3.8)	2.8±0.11 (2.5 to 3.0)	1.15±0.03† (1.10 to 1.21)
As+Mg	4.1±0.34 (3.4 to 5.1)	4.1±0.34† (3.2 to 4.9)	3.9±0.16 (3.6 to 4.2)	2.7±0.18 (2.5 to 2.9)	1.33±0.13† (1.01 to 1.39)
As+Ca	7.3±1.40 (5.0 to 11.35)	2.9±0.21† (2.3 to 3.2)	2.8±0.55 (2.7 to 2.9)	2.8±0.14 (2.6 to 3.1)	1.28±0.15† (1.10 to 1.48)

TBARS - thiobarbituric acid reactive substances; ROS - reactive oxygen species measured in RBC in the first minute; GSH and GSSG - reduced and oxidised glutathione; values are expressed as mean ± SE (N=6); range values are in parenthesis
† - significantly different from all other values; ‡ - significantly different from unmarked values

Table 3 *Effects of selenium, magnesium, and calcium on arsenic-induced oxidative stress in rat liver*

Experimental group	TBARS / $\mu g\ g^{-1}$	ROS / $nmol\ mL^{-1}$	GSH / $\mu mol\ g^{-1}$	GSSG / $\mu mol\ g^{-1}$	AST / $nmol\ mg^{-1}$	ALT / $nmol\ mg^{-1}$	Liver As / $ng\ g^{-1}$
Negative control	4.6±0.47 (3.6 to 5.6)	1.7±0.13 (1.6 to 2.3)	5.9±0.10 (5.6 to 6.1)	4.3±0.17 (3.8 to 4.6)	10.9±0.18 (8.6 to 11.1)	10.87±0.12 (10.1 to 11.4)	0.1±0.04 (0.5 to 1.4)
As	6.3±0.90[†] (4.4 to 7.8)	2.9±0.22[†] (1.9 to 3.8)	3.9±0.16[†] (3.7 to 4.3)	5.1±0.32 (4.6 to 6.5)	16.1±1.08[†] (12.8 to 19.6)	19.86±0.83[†] (15.9 to 22.5)-	3.3±0.11[†] (2.5 to 3.9)
As + Se	4.7±0.48 (3.5 to 5.8)	1.8±0.13 (1.5 to 2.1)	5.1±0.30 (4.7 to 6.0)	4.7±0.40 (3.8 to 5.4)	12.0±0.38[‡] (10.8 to 13.7)	13.14±0.43[‡] (11.4 to 15.1)	2.1±0.18[‡] (1.7 to 2.4)
As + Mg	6.0±0.23[†] (5.0 to 7.4)	2.2±0.15[‡] (1.7 to 2.4)	6.5±0.34 (6.0 to 7.5)	4.8±0.14 (4.2 to 5.2)	12.6±0.21[‡] (11.3 to 14.2)	12.16±0.14[‡] (11.6 to 13.4)	3.5±0.32[†] (2.2 to 4.3)
As + Ca	5.8±0.16[†] (4.0 to 7.1)	2.7±0.40[†] (2.0 to 3.8)	7.3±0.20 (6.9 to 7.6)	4.8±0.12 (4.4 to 5.1)	12.3±0.25[‡] (11.4 to 12.9)	16.79±0.17[§] (15.4 to 18.1)	3.2±0.13[†] (2.7 to 3.5)

TBARS - thiobarbituric acid reactive substances; ROS - reactive oxygen species measured in RBC in the first minute; GSH and GSSG - reduced and oxidized glutathione; AST and ALT - alanine and aspartate transaminases measured in protein in the first minute; values are expressed as mean ± SE (N=6); range values are in parenthesis
† - significantly different from all other values; ‡ and § - significantly different from unmarked values

selenium, magnesium and calcium to counteract these changes. Our results single out selenium as the most effective protection against arsenic-induced oxidative stress. Selenium is an essential trace element, but at higher doses it is toxic. It is a component of antioxidant enzyme glutathione peroxidase, which together with superoxide dismutase (SOD), catalase (CAT), and vitamin E neutralises ROS. Selenium is known to affect the distribution of many toxic metals. Arsenic has been reported to increase hepatobiliary transport of selenium and facilitate its accumulation in RBCs, and selenite, in turn, to increase biliary excretion of arsenic (3, 22, 23). Glattre et al. (24) studied the distribution and interaction between arsenic and selenium in rat thyroid. Rats receiving arsenic plus selenium had the same concentrations of both elements as those measured in the groups pre-treated with arsenic or selenium alone. This suggests that both arsenic and selenium accumulate in the thyroid tissue. Post-mortem examination of the thyroid following arsenic exposure showed reduction in size and histological changes in thyroid follicles, whereas only minor changes were observed in selenium or arsenic-plus-selenium treated group. This competition between selenium and toxic metals was contributed to functional proteins and bioligands or active tissue sites or to formation of a reversible compound, metal

selenide, which reduced "free" toxic metal ions in the body. Metal selenide may result from interaction between a metal and active selenium (selenide) released from sodium selenite *in vivo* (18). Badiello et al. (25) suggested that selenium could detoxify metal ions, in addition to its antioxidant action. The authors consider dietary selenium supplementation necessary, preferably in the form of selenomethionine.

Metals and calcium interact at several sites in the body, including cell membrane (through mechanisms that regulate ion transport) (26). Calcium deficiency is known to decrease lead clearance and increases lead absorption, whereas excess calcium only slightly decreases lead clearance and has little effect on lead absorption (27). The mechanism by which calcium interferes with arsenic absorption is not clear. However, several interesting studies suggest that calcium intake rather than calcium status modulates metal absorption in animals (28). Calcium appears to partly inhibit metal absorption via competition for common binding sites on intestinal binding proteins (29).

Relative sensitivity of ALAD to arsenic has been reported earlier (30). In this study, we observed significant depletion of ALAD upon arsenic exposure, perhaps due to the binding of As (III) to the essential -SH group. A decrease in ALAD activity is usually

related to inadequate diet, particularly deficient in essential metals like selenium (22). In our study, we found a significant recovery in ALAD activity in the arsenic-exposed rats concomitantly receiving selenium, which might be due to preferential binding of selenium to proteins. We did not observe significant changes in GSH concentration. GSH is a tripeptide of three amino acids (glutamic acid, cysteine, and glycine) and is involved in various physiological and metabolic functions. It is a major inter-organ transporter of cysteine, which has a nucleophilic thiol group important for detoxification of electrophilic metabolites. Liver supplies constituents for GSH synthesis in the kidney and intestine. In this study, increased liver and kidney GSH shows antioxidant action of the trace metals. Mishra et al. (30) also reported a significant decline in GSH concentration following arsenic exposure. They also observed a decrease in the GSH:GSSG ratio as an indicator of oxidative stress in the liver and kidney. Administration of calcium and magnesium in our study did not increase blood GSH concentration (Table 2), and GSH depletion may also explain the hepatotoxic effects of arsenic (Table 3). A decrease in GSH and increase in GSSG levels is a sensitive indicator of oxidative stress, and our study clearly shows that arsenic may be the culprit, possibly due to a disruption of oxidant/antioxidant balance. Arsenic-induced TBARS production in our study also supports the oxidative stress hypothesis. As selenium reduced elevated TBARS level, it seems to play a vital role in protecting cells from oxidative stress.

This protective role of selenium is further corroborated by AST and ALT activities, indicative of liver damage. All three trace metals were effective in reducing the level of transaminases in arsenic-exposed animals.

Our study has shown that trace metals are valuable against arsenic exposure, selenium in particular if administered at a low level. However, most of the observed changes were only marginal and of much smaller magnitude than the effects of chelation therapy. We therefore recommend that selenium is added to chelation therapy to achieve the best effect.

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Sažetak

ZAŠTITNO DJELOVANJE SELENA, KALCIJA I MAGNEZIJA PROTIV OKSIDATIVNOGA STRESA UZROKOVANOG ARSENOM U MUŽJAKA ŠTAKORA

Anorganski arsen snažan je kancerogen i onečišćivač okoliša. Više od stotinu milijuna ljudi izloženo je povišenim koncentracijama arsena, ponajviše u pitkoj vodi. Esencijalni elementi u tragovima mogu utjecati na toksičnost metala na primarnome mjestu djelovanja te na drugačiji odgovor tijela na toksične metale tako što mijenjaju njihov metabolizam i prijenos. Namjera ovoga istraživanja bila je utvrditi utjecaj istodobne primjene selena, magnezija i kalcija s arsenom na neke biokemijske parametre u krvi te na oksidativni stres. Selen primijenjen istodobno s arsenom najdjelotvornije je smanjio inhibiciju dehidrataze delta-aminolevulinske kiseline (ALAD) u krvi te oksidativni stres u jetri. I djelovanje kalcija i magnezija pokazalo se povoljnim na neke hematološke i biokemijske parametre. Ovi rezultati upućuju na obećavajuću ulogu selena u sprječavanju nastanka znakova i simptoma povezanih s izlaganjem arsenu.

KLJUČNE RIJEČI: *ALAD, elementi u tragovima, GSH, ROS, TBARS*

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